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A uniformly aligned multilayer of membranes containing peptides has one-dimensional structural order in which the bilayers are the unit cells and preserves the orientational order of peptides relative to the plane of membrane. Our goal is to develop methods to extract these structural information, and use such methods to study the structural bases of the voltage-gating mechanisms in model channels. In the past year, we have developed the method of oriented circular dichroism, by which we can indeed extract the orientational information of helical peptides in membrane. We have also found that our multilayer samples produce high resolution diffraction data, from which we can obtain the one-dimensional electron density profiles of peptides in bilayer membranes, in particular the position of heavy atomic ions. This report describes the application of these methods to study the voltage-gating mechanism of the alamethicin channel and the location of ion binding sites of the gramicidin channel.

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ANNUAL REPORT ON GRANT N00014-90-J-1020

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GRANTEE: Rice University
GRANT TITLE: Investigating the Structural Bases of Voltage-gated Model Channels by Using Perfectly Aligned Multilayer Samples

INTRODUCTION

It is clear that membrane proteins need to be studied with all available probes. In this project, we choose to study channel-forming peptides in uniformly aligned multilayer membranes. This system has one-dimensional structural order in which the bilayers are the unit cells and contains the orientational order of peptides relative to the plane of membrane. Our goal is to develop methods to extract these structural information, and use such methods to study the structural bases of the voltage-gating mechanisms in model channels.

In the past year, we have developed the method of oriented circular dichroism (Wu, Huang & Olah, 1990), by which we can indeed extract the orientational information of helical peptides in membrane. We have also found that our multilayer samples produce high resolution diffraction data, from which we can obtain the one-dimensional electron density profiles of peptides in bilayer membranes, in particular the position of heavy atomic ions. We have applied these methods to study alamethicin and gramicidin.

VOLTAGE-GATING MECHANISM OF ALAMETHICIN

Although the voltage-dependent alamethicin channel is one of the best characterized ion channels, so far no agreement has been reached about which model best describes all the experimental data. While the barrel-stave configuration is accepted by most investigators as a good description of the conducting state of alamethicin, there are conflicting reports on its nonconducting state--in the absence of an applied field, some found alamethicin molecules on the membrane surface, but others found them incorporated in the hydrophobic core of the membrane. This problem is now resolved by the discovery of a phase transition of alamethicin in membrane. We have discovered that, as a function of lipid/peptide ratio L/P and the chemical potential of water μ, alamethicin molecules are either all bind parallel to the membrane surface or all insert perpendicularly into the membrane. The state of alamethicin was monitored by the method of oriented circular dichroism, using aligned multilayer samples in the liquid crystalline L_α phase (Fig.1). If L/P exceeds a critical value, all peptide molecules are on the membrane surface. If L/P is below the critical value, all peptide molecules are incorporated in the membrane when μ is high; when μ is low, alamethicin is again on the membrane surface (Fig.2). In a typical conduction experiment, alamethicin molecules are partitioned between the aqueous phase and the lipid phase;

in the lipid phase, the lipid/peptide ratio is such that all alamethicin molecules are on the membrane surface in the absence of a field. When an electric field is applied, it is those surface peptide molecules (rather than those in the aqueous phase) which will probabilistically turn into the membrane to form channels. The phase transition is a manifestation of membrane-mediated intermolecular interactions between peptide molecules. It can be qualitatively explained in terms of a model (Huang and Wu, 1990).

LOCATION OF ION BINDING SITES IN THE GRAMICIDIN CHANNEL

This is the first x-ray diffraction on gramicidin in its membrane-active form. High-resolution Bragg reflections of uniformly aligned multilayer samples of membranes containing gramicidin and ions (Tl^+, K^+, Ba^{++}, Mg^{++} or without ions) are obtained. From the difference electron density profiles (Figs. 3-6), we found a pair of symmetrically located ion binding sites for Tl^+ at 9.6±0.3Å and for Ba^{++} at 13.0±0.2Å from the midpoint of the gramicidin channel. The location of Ba^{++} binding sites is near the ends of the channel, consistent with the experimental observation that divalent cations do not permeate but block the channel. The location of Tl^+ binding sites is somewhat a surprise. It was generally thought that monovalent cations bind to the first turn of the helix from the mouth of the channel. (It is now generally accepted that the gramicidin channel is a cylindrical pore formed by two monomers, each a single-stranded $\beta^{6.3}$ helix and hydrogen-bonded head-to-head at their N-termini.) But our experiment shows that the Tl^+ binding site is either near the bottom of or below the first turn of the helix. (Olah, Huang, Liu, and Wu, 1990)

FIGURE LEGENDS

Fig. 1 Oriented circular dichroism (OCD) of an aligned multilayer sample of DPhPC/alamethicin molar ratio 50/1 when the sample is in equilibrium with 100% RH (spectra I) and with 50% RH (spectra S). CD was measured with light incident at an angle α relative to the normal to the planes of bilayers. The α-dependence of spectra I indicates that the helical parts of alamethicin molecules are perpendicular to the plane of bilayer, whereas the α-dependence of spectra S indicates that the helices are parallel to the plane of bilayer. The solid lines for the α=0° spectra are the least-squares fits; the solid lines for the spectra of oblique angles are theoretical constructions from the α=0° spectra (Wu, Huang and Olah, 1990).

Fig. 2 The phase diagram for alamethicin in DPhPC on the plane of relative humidity (RH) versus the lipid/peptide molar ratio (L/P). A multilayer sample of a certain L/P was in turn equilibrated in humidity chambers of various RH; in each equilibrium state, its OCD was measured. If the OCD are spectra I (Fig. 1), indicating that alamethicin is in the inserted state, an open circle is shown at the corresponding L/P and RH. If the OCD are spectra S,

indicating that alamethicin is in the surface state, a black circle is shown. A gray circle implies that the OCD are linear superpositions of spectra I and spectra S, indicating that the state of alamethicin is a coexistent state. The shaded area for L/P=10/1 indicates that the sample at RH below 89% turned into the gel phase. In all other data points, the samples were in the L_α phase. We define a critical value of L/P, L/P*. For L/P greater than L/P*, the alamethicin is always in the surface state; for L/P small than L/P*, the alamethicin is always in the inserted state if the sample is in equilibrium at 100% RH. (Huang and Wu, 1990)

Fig. 3 Normalized electron density profiles of gramicidin/DLPC bilayers with Tl^+ (dotted line), with K^+ (dashed line) and without salt (solid line), all at the lamellar spacing 43.4 Å. (Olah, Huang, Liu and Wu, 1990)

Fig. 4 Difference electron density profiles. The top two are ρ(thallium sample)-ρ(salt free sample). The bottom two are ρ(thallium sample)-ρ(potassium sample). Solid lines are obtained from the profiles of lamellar spacings 43.4 Å; dotted lines from lamellar spacing 42.4 Å. (Olah, Huang, Liu, and Wu, 1990)

Fig. 5 Normalized electron density profiles of gramicidin/DLPC bilayers with Ba^{++} (dotted line) and with Mg^{++} (solid line), at lamellar spacing 42.8 Å. (Olah, Huang, Liu, and Wu, 1990)

Fig. 6 Difference electron density profiles ρ(barium sample)-ρ(magnesium sample) at lamellar spacing 42.8 Å and 44.4 Å. (Olah, Huang, Liu, and Wu, 1990)

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2. Olah, G. A., Huang, H. W., Liu, W. and Wu, Y. (1990) Location of Ion Binding Sites in the Gramicidin Channel by X-ray Diffraction. submitted to J. Mol. Biol.

3.Huang, H. W. and Wu, Y. (1990) Alamethicin-membrane Interactions and Voltage-gating Mechanism of Alamethicin Channel. submitted to Biophys. J.

4. Olah, G. A. (1990) Tl^+ Distribution in the Gramicidin Ion Conducting Channel Determined by X-ray Diffraction. Ph.D. Thesis, Rice University.

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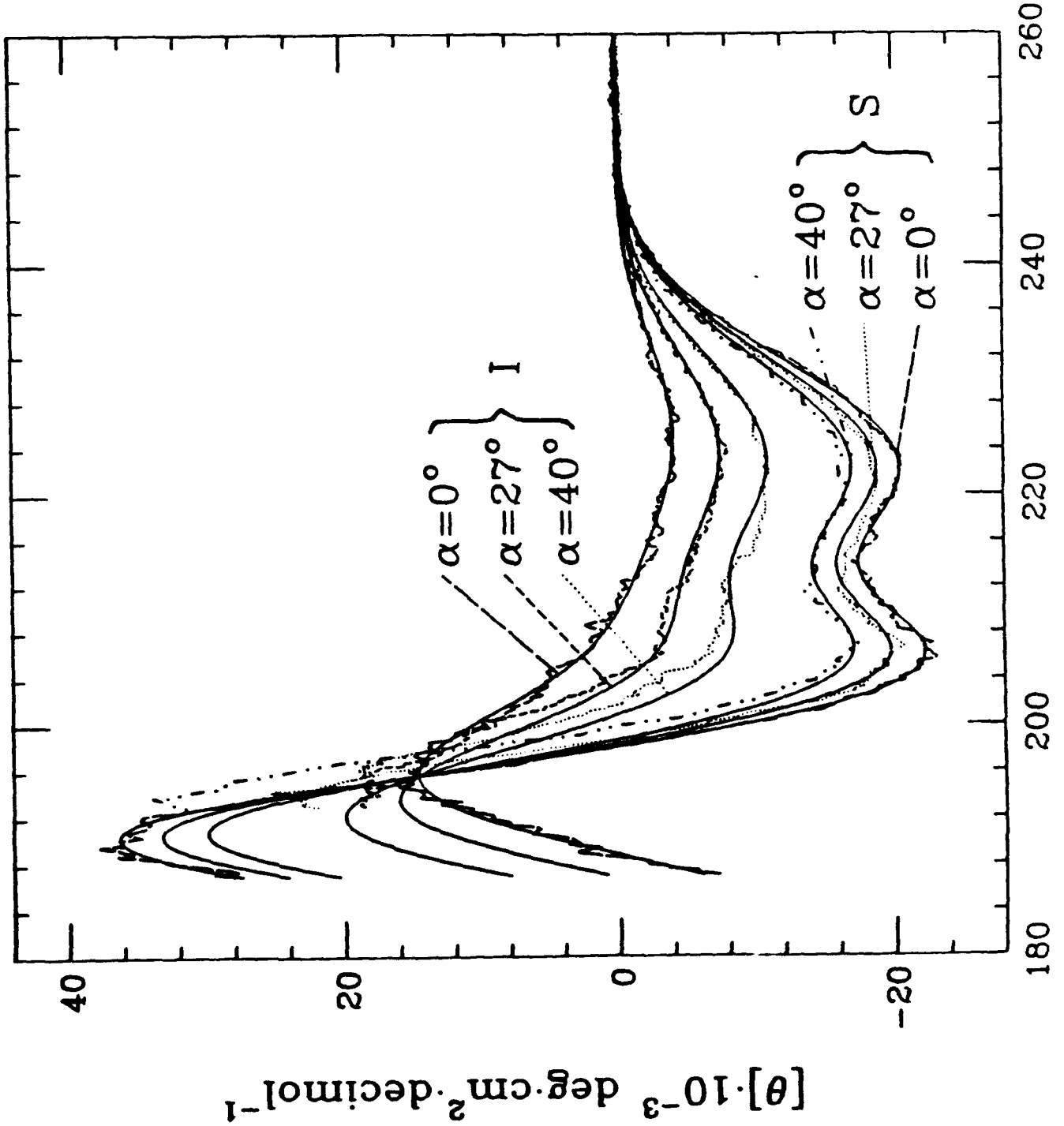
[θ]·10⁻³ deg·cm²·decimol⁻¹
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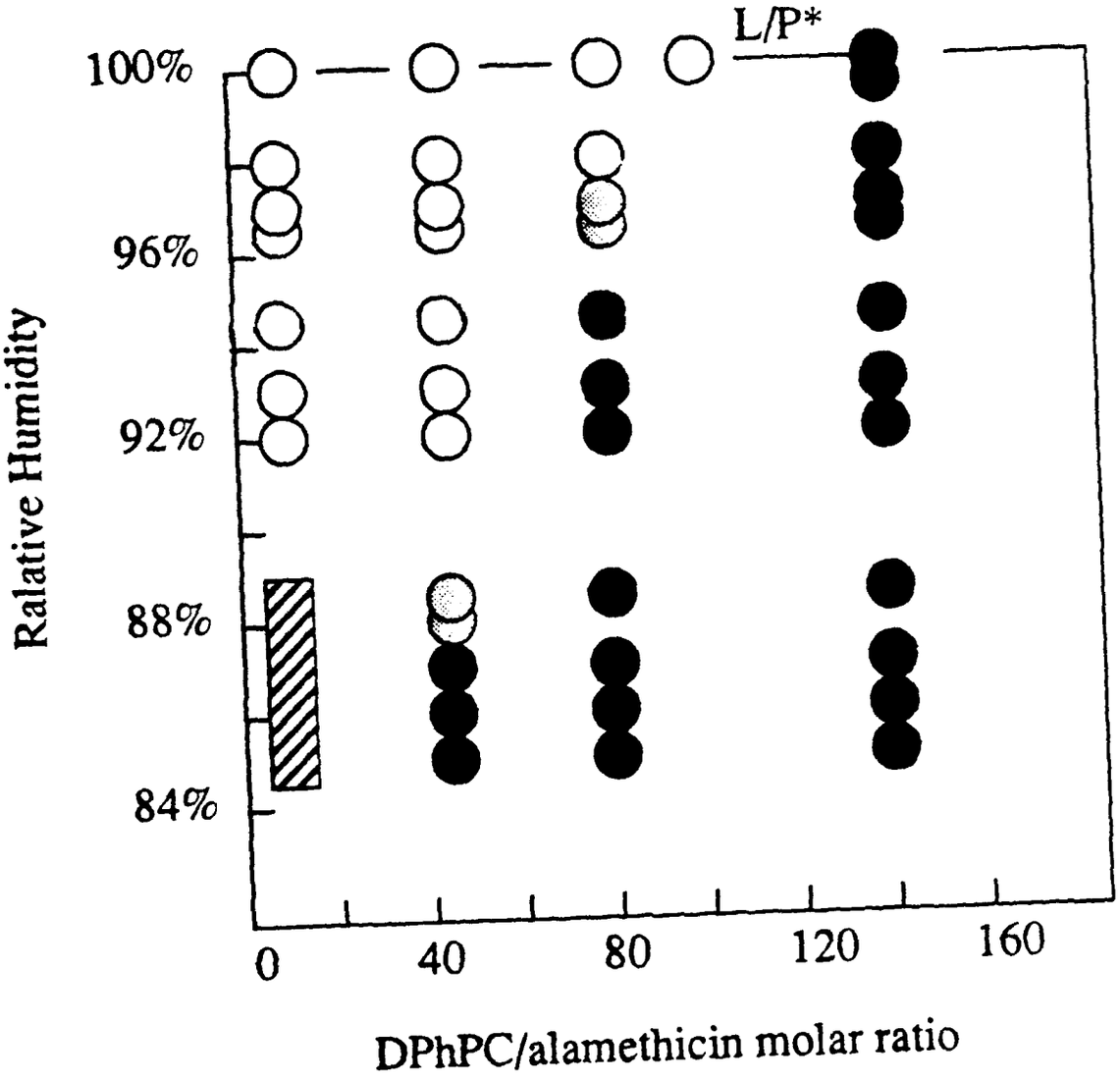
20
0
-20
180
200
220
240
260
wavelength (nm)
α=0°
α=27°
α=40°
I
α=40°
α=27°
α=0°
S

Figure 1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88%
84%
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80
120
160
DPhPC/alamethicin molar ratio

Figure 2

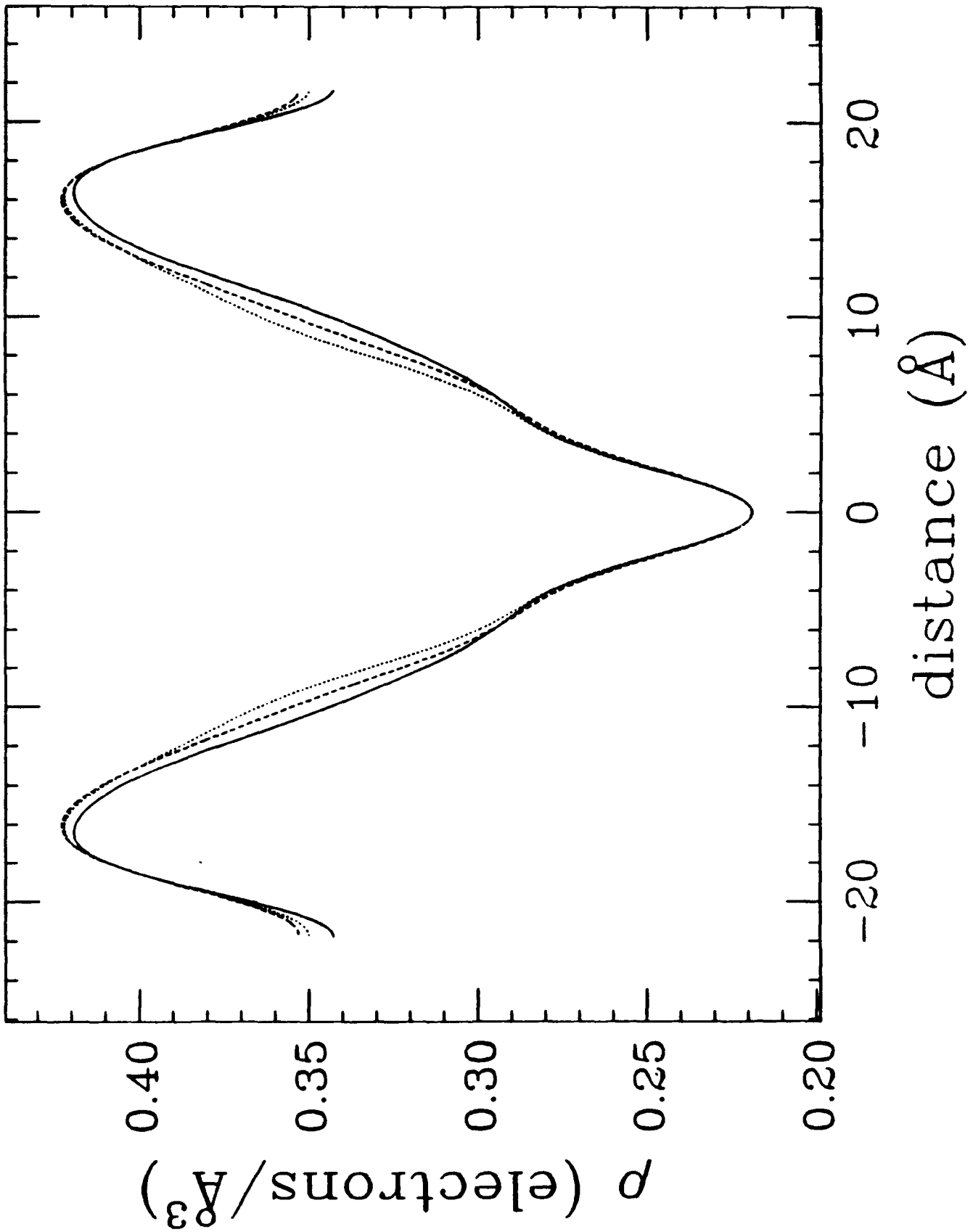
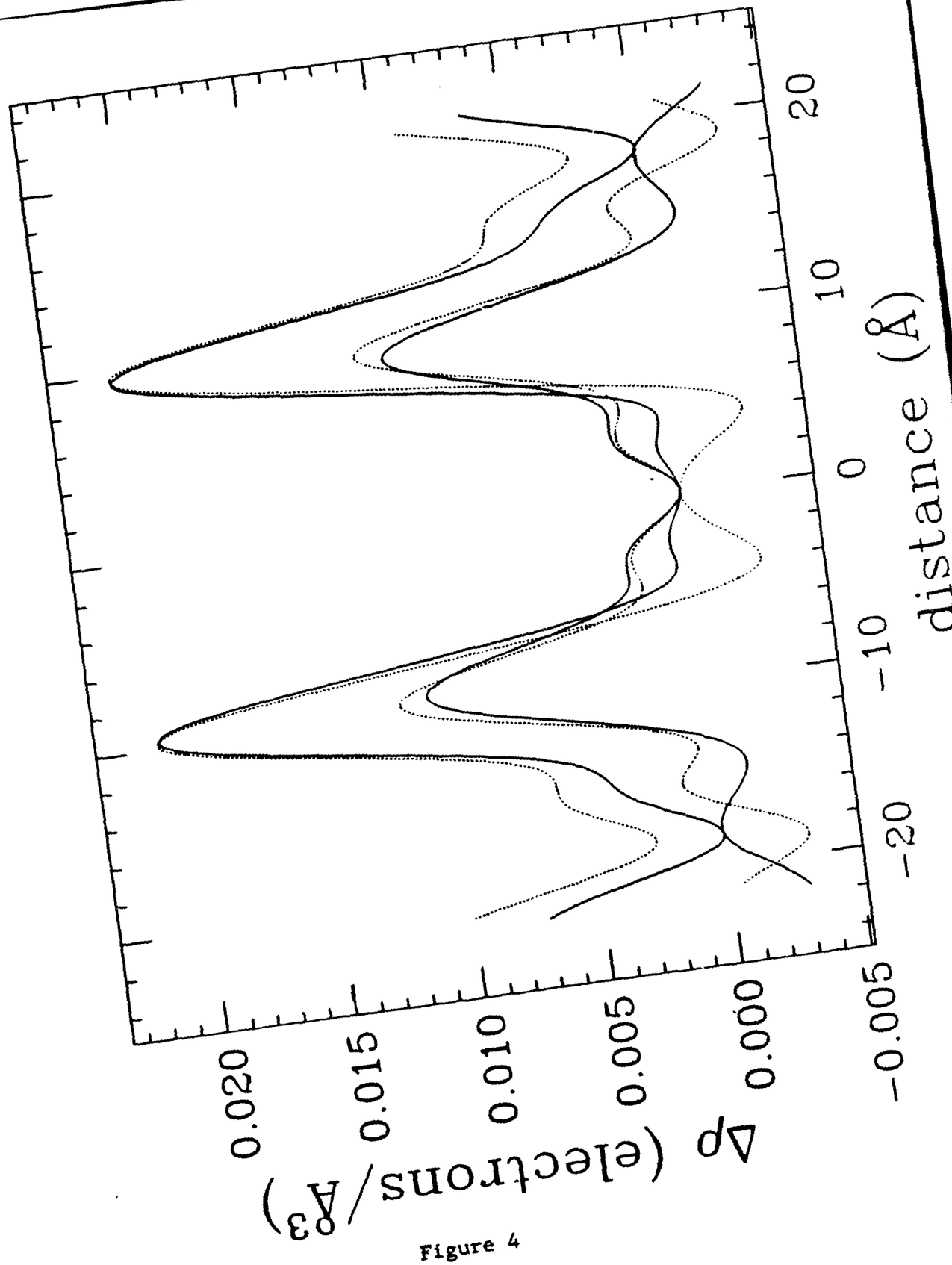


Figure 3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-0.005
distance (Å)
20
10
0
-10
-20

Figure 4

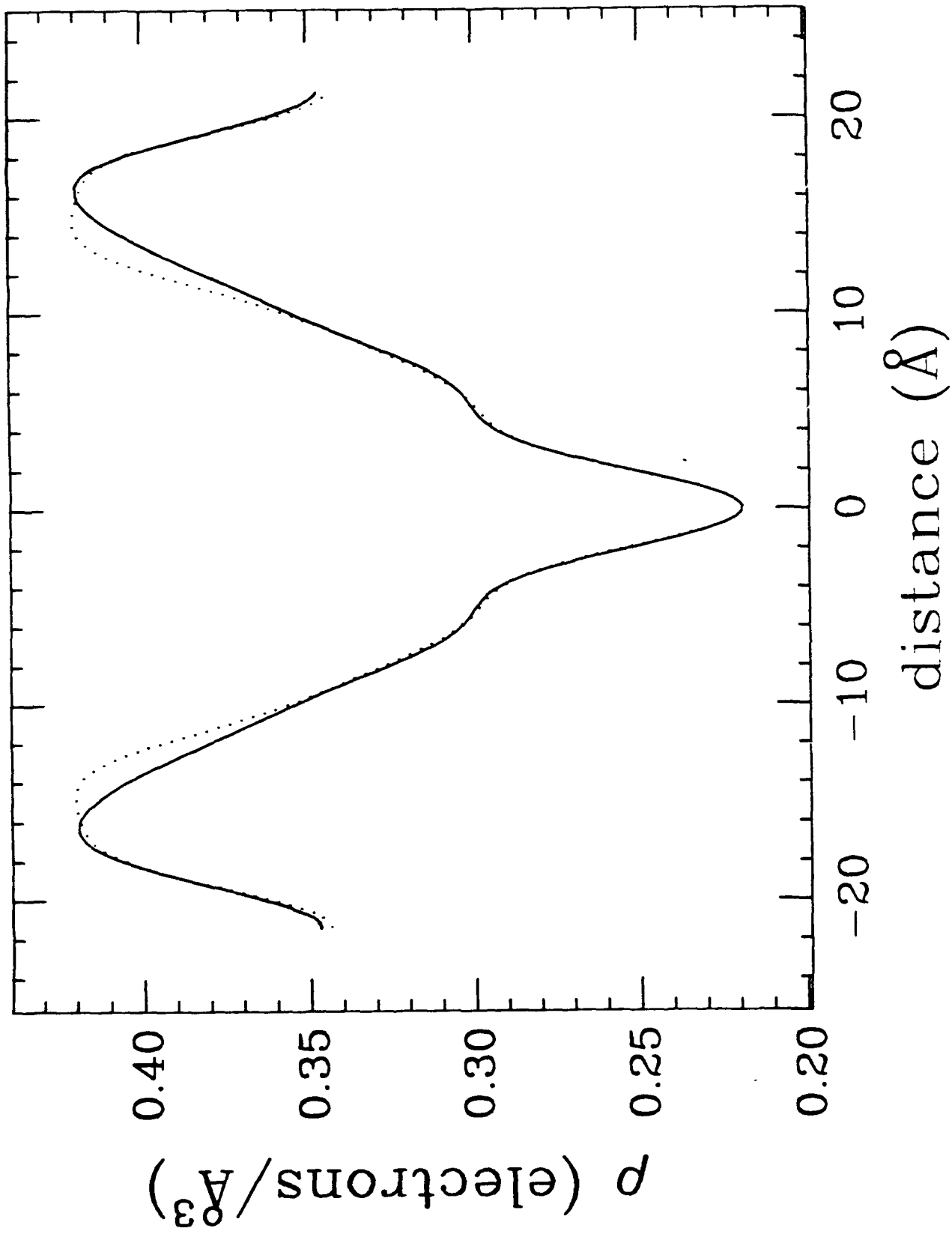

Figure 5

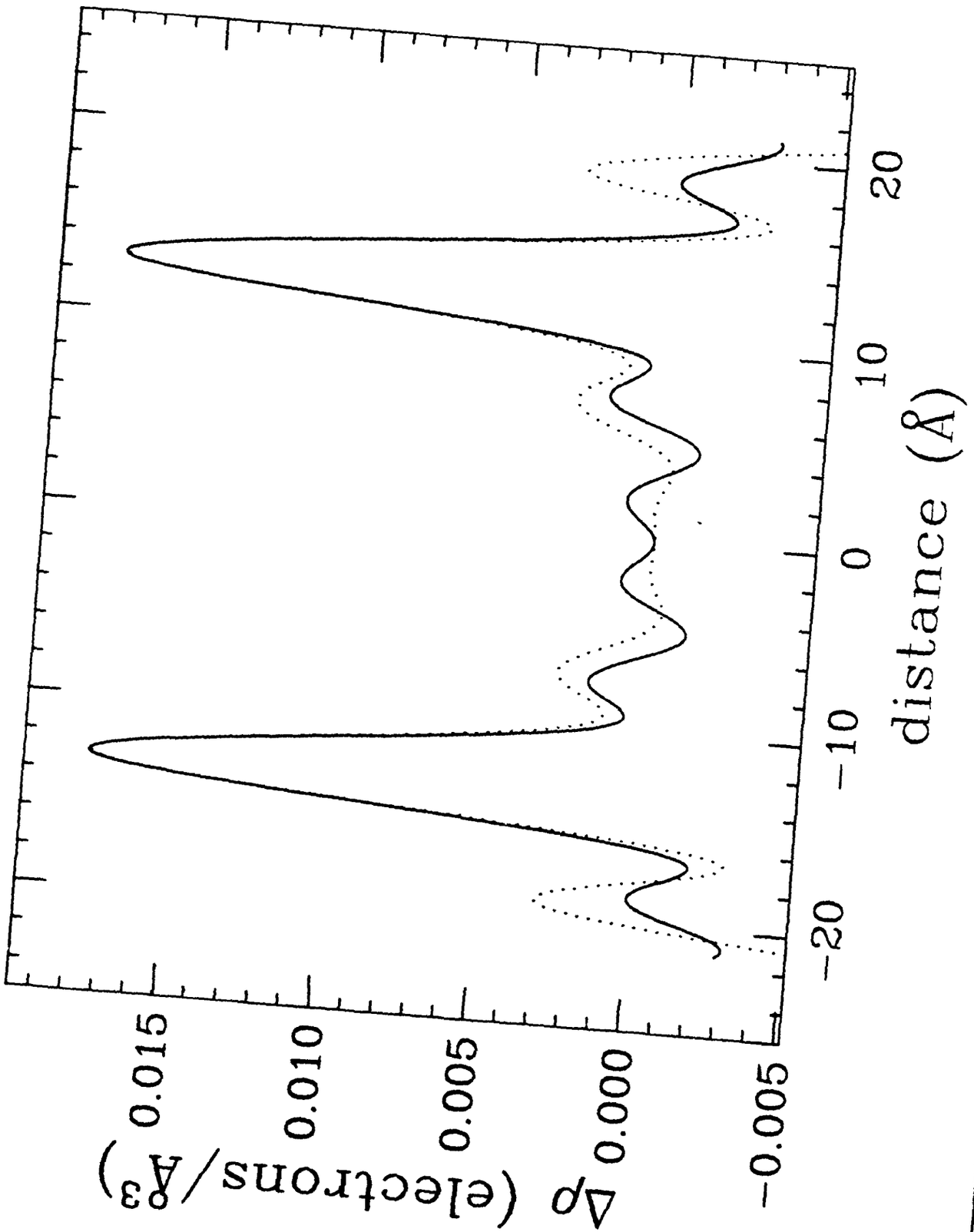
0.015
0.010
0.005
0.000
−0.005
Δρ (electrons/Å³)
−20
−10
0
10
20
distance (Å)

Figure 6

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